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Probing the Secondary Structure of Membrane Proteins with the Pulsed EPR ESEEM Technique

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A novel approach is being developed to probe the secondary structure of membrane proteins and peptides qualitatively utilizing the three-pulse Electron Spin Echo Envelope Modulation (ESEEM) pulse sequence. In order to demonstrate the practicality of this biophysical technique, the M2delta subunit of AChR (α-helical), KAGAKI (β-sheet) and VRL8 (310-helix) peptides were incorporated into phospholipids bicelles to probe their secondary structure with ESSEM spectroscopy. Utilizing site-directed spin-labeling (SDSL) coupled with deuterated amino acid labeling of the peptides, the corresponding ESEEM spectra reveal characteristic patterns for α-helix, β-sheet and 310-helical structures. This ESSEM secondary structural approach can be used with different deuterated amino acids and provide pertinent qualitative structural information on membrane proteins in a short period of time (10 minutes) with small amounts of sample (30 μg).

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Site-Directed Spin Labelling of Sulfite Oxidase using Non Natural Amino Acids

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Site-directed spin labeling (SDSL) in combination with EPR spectroscopy has proven to be a valuable tool to investigate structure and dynamics of proteins. Commonly, spin labels are introduced into proteins using cysteine residues. However, this strategy fails if proteins contain functionally important cysteines. A recent proof-of-principle study using T4 Lysozyme as a model system has shown that non-natural p-acetylphenylalanine binding the spin label through a ketoxime ligation can be used for SDSL [1]. While T4 Lysozyme does not contain essential amino acids and is a rather stable protein, the current contribution will present a study on sulfite oxidase, a molybdo-enzyme, which carries an essential cysteine residue binding the Moco-cofactor, using the same spin labeling strategy as in [1].

Within this contribution we will discuss challenges involved in the labeling of a more fragile protein using the less reactive ketoxime ligation in comparison to the disulfide linkage and will explore the information gained from the EPR line shapes of these spin labels in terms of structure determination, using mutations along a helical turn as an exploratory example. In addition, doubly spin labeled proteins were created to determine distances between the spin labels using pulsed electron-electron double resonance (pELDOR/DEER) experiments. The distance distributions extracted from these measurements will be discussed in light of expectations based on simple geometric considerations and will be compared to results of cysteine based distance determinations.

[1] M. R. Fleissner, E. M. Brustad, T. S. Kalai, C. Altenbach, D. Cascio, F. B. Peters, K. L. N. Hideg, S. Parker, et al., Site-directed spin labeling of a genetically encoded unnatural amino acid, Proc. Nat. Acad. Sci. 2009, 106, 21637.

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High-Resolution Measurement of Distance and Orientation in Myosin: EPR of a Bifunctional Spin Label

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We present a method for obtaining high-resolution information on protein backbone structure and dynamics using electron paramagnetic resonance (EPR) of a bifunctional spin label (BSL) and molecular modeling. Two complementary EPR techniques were employed to measure protein orientation (conventional EPR) and intra-protein distances (dipolar electron-electron resonance, DEER). BSL attaches at Cys positions *i* and *i*+4 on a helix, greatly reducing probe mobility relative to the peptide backbone, compared to monofunctional labels. Accurate modeling of BSL provides the coordinates required to directly relate spectroscopic data to backbone structure (both orientation and distance), and dynamics (rotational motion). In the current work, the motor protein *Dictyostelium* myosin II was used to demonstrate this approach. We measured nucleotide-dependent structural transitions of two key helices within the myosin catalytic domain (CD). Two double-Cys sites were engineered, with one Cys pair located on the relay helix, and the other on a stable helix in the upper 50kD domain. BSL on a construct with one of these pairs was used to measure myosin orientation relative to oriented actin. BSL on a construct with two pairs was used to measure interprobe distances. The effect of ADP binding on both orientation and distance was clearly detected with BSL, but not with a monofunctional label. The significance of this work is twofold: (1) A structural transition in the relay helix upon ADP binding was clearly defined with high resolution. (2) BSL spectra demonstrate superior resolution, compared to monofunctional spin labels, making it possible to directly translate spectroscopic data to protein structure and dynamics. This work was funded by grants from NIH (R01 AR32961, T32 AR07612, P30 AR0507220).

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Dynamic and Contrasting Information by Oriented-Sample Solid-State NMR Spectroscopy of Membrane Proteins

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Oriented-sample NMR (OS NMR) has emerged as a powerful technique for structure determination of membrane proteins in their native-like lipid environment. We have developed a model relating OS NMR lineshapes to uniaxial ordering (mosaic spread) and rotational diffusion of the protein within the membrane. The model is exemplified by 15N NMR spectra of Pf1 coat protein in both magnetically aligned phage and reconstituted in oriented bicelles. In the case of Pf1 phage, the lineshapes are dominated by static uniaxial disorder; whereas fast rotational diffusion of the protein is responsible for the motional line narrowing in perpendicularly oriented bicelles. From the analysis of 15N NMR linewidths, rotational diffusion coefficient can be estimated. Since the value of the diffusion coefficient is ultimately related to the overall protein size, information about oligomerization states is potentially obtainable. Second, the use of various membrane-embedded radicals allows one to both dramatically speed up data acquisition, on the one hand, and obtain contrasting information for membrane-embedded proteins, on the other. While membrane-bound paramagnetic species drastically affect the T1Z relaxation times (at 2:1 molar ratio relative to the protein), the transverse T2 relaxation is only slightly affected. 5-DOXYL stearic acid, TEMPOL, and CAT-1 radicals exhibit different partitioning within the membrane, and result in differential paramagnetic effect on the spectral peaks arising from different residues of Pf1 protein in bicelles. This allows one to obtain contrasting information about the location of the residues relative to the membrane. As was shown by EPR, TEMPOL partitions itself equally in and out of the membrane, and almost uniformly affects all residues within the bilayer. By contrast, 5-DOXYL stearic acid affects mostly the residues below the interfacial region, while CAT-1 affects the residues located within the polar head groups.

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Structure and Function of Bacterial Biofilms by Solid-State NMR

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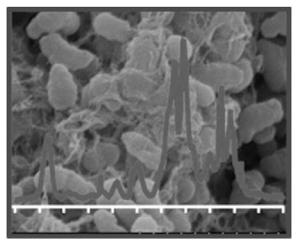
Biofilms are multi-cellular communities formed by bacteria, and they consist of bacteria encased within a non-crystalline extracellular matrix (ECM) of proteins, polysaccharides, and small molecules. Biofilm formation provides increased protection of bacteria from antibiotics and host defenses. New quantitative approaches are needed to define composition and architecture of biofilms. We have utilized solid-state NMR to quantitatively define the composition of the wild-type uropathogenic E. coli UTI89 extracellular matrix. Recently, we have extended the same methodology to elucidate changes in biofilm composition due to inhibitory small molecules and to define the composition of Vibrio cholerae biofilm.



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NMR Structural Studies of Antimicrobial Peptides as In-Plane Helix of Membrane Proteins

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Antibiotics from organic compounds are a long established part of our defense against bacterial pathogens. Their wide use has led to pathogens' increased